EXPRESS MAIL NO. EV576489826US		
DECLARATION OF KARL WEISGRABER UNDER 37 C.F.R. § 1.132 Address to: Commissioner for Patents Alexandria, VA 22313-1450	Attorney Docket Confirmation No.	UCAL-222 5282
	First Named Inventor	Karl H. Weisgraber
	Application Number	10/017,718
	Filing Date	December 14, 2001
	Group Art Unit	1632
	Examiner Name	T.N. Ton
	Title	*Gene-targeted animal model of apolipoprotein E4 domain interaction and uses thereof*

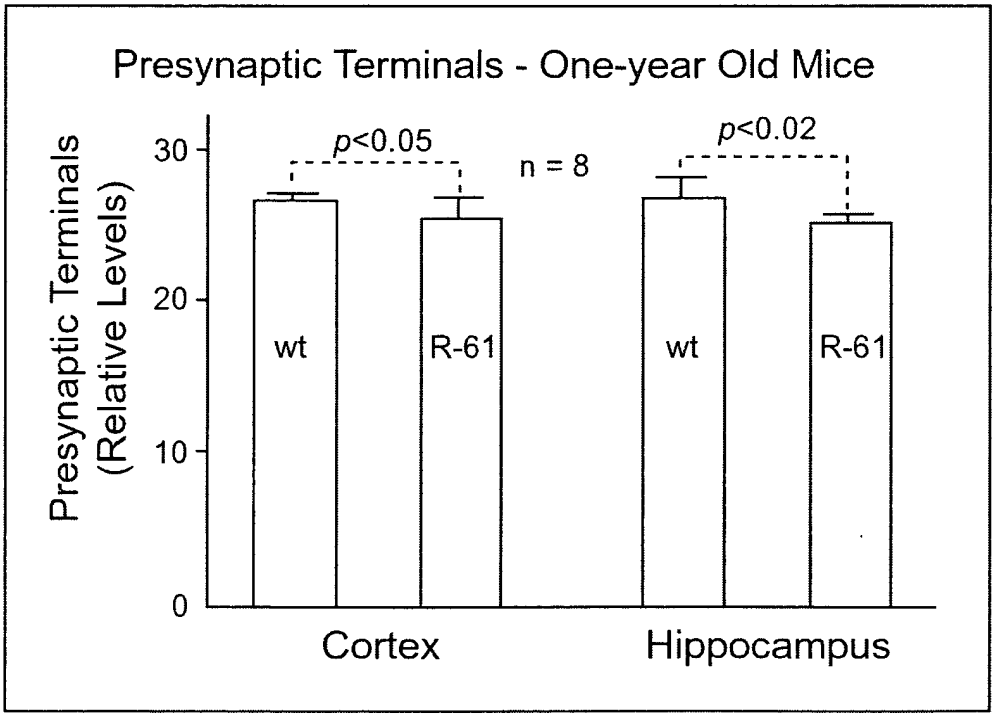
Dear Sir:

1. I, Karl Weisgraber, declare and say I am a co-inventor of the claims of the above-identified patent application.

2. I have read the Office Action dated October 1, 2004 in this application and understand that the Examiner would like to see further evidence that a gene-targeted mouse that produces an Arg-61 modified apoE exhibits a phenomenon associated with Alzheimer's Disease (AD).

3. The following paragraphs describe experiments conducted in my laboratory. The results of the experiments provide further evidence for the fact that a gene-targeted mouse that bears a Thr→Arg substitution at a position equivalent to Arg-61 in human apoE4 exhibits a phenomenon associated with AD, and therefore is suitable for use in identifying agents that reduce a phenomenon associated with AD.

4. Assessment of neurodegeneration in wild type (wt) and Arg-61 (R-61) mice by examination of presynaptic terminal density. Fixed hemi-brains from one-year mice were vibratome sectioned and the tissue sections were immunolabeled with a monoclonal antibody specific for synaptophysin (presynaptic terminals). The labeled sections were examined by confocal microscopy and computer-assisted analysis was used to quantitate the level of immunoreactive presynaptic terminals in the cortex and hippocampus, a validated method to measure neuronal integrity. The results, depicted in the Figure below, demonstrate that there is a significant decrease in the presynaptic terminal density in the Arg-61 mice compared to wt mice, indicating significant neurodegeneration in the one-year old Arg-61 mice.



5. This study provides a direct link of apoE domain interaction with neurodegeneration, which is a phenomenon associated with AD.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such will false statements may jeopardize the validity of the application or any patent issuing thereon.

2/22/05
Date

Karl H. Weisgraber
Karl H. Weisgraber

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Exhibit 2
U.S. Patent No. 10/017,718

1: Proc Natl Acad Sci U S A. 1993 Mar 1;90(5):1977-81.

Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease.

Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghil J, Salvesen GS, Roses AD.

Department of Medicine (Neurology), Joseph and Kathleen Bryan Alzheime Disease Research Center, Duke University Medical Center, Durham, NC 27710.

Apolipoprotein E is immunochemically localized to the senile plaques, vascular amyloid, and neurofibrillary tangles of Alzheimer disease. In vitro, apolipoprotein E in cerebrospinal fluid binds to synthetic beta A4 peptide (th primary constituent of the senile plaque) with high avidity. Amino acids 12-2 of the beta A4 peptide are required. The gene for apolipoprotein E is located chromosome 19q13.2, within the region previously associated with linkage o late-onset familial Alzheimer disease. Analysis of apolipoprotein E alleles in Alzheimer disease and controls demonstrated that there was a highly significant association of apolipoprotein E type 4 allele (APOE-epsilon 4) an late-onset familial Alzheimer disease. The allele frequency of the APOE-epsilon 4 in 30 random affected patients, each from a different Alzheimer disease family, was 0.50 +/- 0.06; the allele frequency of APOE-epsilon 4 in age-matched unrelated controls was 0.16 +/- 0.03 ($Z = 2.44$, $P = 0.014$). A functional role of the apolipoprotein E-E4 isoform in the pathogenesis of late onset familial Alzheimer disease is suggested.

PMID: 8446617 [PubMed - indexed for MEDLINE]

Exhibit 3
U.S. Patent No. 10/017,718

1: Neurology. 1993 Aug;43(8):1467-72. Related Articles, Li

Comment in:
- Neurology. 1994 Dec;44(12):2420-1.
- Neurology. 1994 Dec;44(12):2420; author reply 2421.

Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease.

Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ, et al.

Department of Medicine [Neurology], Joseph and Kathleen Bryan Alzheimer Disease Research Center, Duke University Medical Center, Durham, NC.

Apolipoprotein E, type epsilon 4 allele (APOE epsilon 4), is associated with late-onset familial Alzheimer's disease (AD). There is high avidity and specif binding of amyloid beta-peptide with the protein ApoE. To test the hypothesi that late-onset familial AD may represent the clustering of sporadic AD in families large enough to be studied, we extended the analyses of APOE allel to several series of sporadic AD patients. APOE epsilon 4 is significantly associated with a series of probable sporadic AD patients (0.36 +/- 0.042, AI versus 0.16 +/- 0.027, controls [allele frequency estimate +/- standard error], = 0.00031). Spouse controls did not differ from CEPH grandparent controls from the Centre d'Etude du Polymorphisme Humain (CEPH) or from literatu controls. A large combined series of autopsy-documented sporadic AD patie also demonstrated highly significant association with the APOE epsilon 4 all (0.40 +/- 0.026, p < or = 0.00001). These data support the involvement of ApoE epsilon 4 in the pathogenesis of late-onset familial and sporadic AD. ApoE isoforms may play an important role in the metabolism of beta-peptide and APOE epsilon 4 may operate as a susceptibility gene (risk factor) for the clinical expression of AD.

PMID: 8350998 [PubMed - indexed for MEDLINE]

Exhibit 4
U.S. Patent No. 10/017,718

1: Am J Cardiol. 1993 Jan 15;71(2):160-5. Related Articles, Li

Relation of apolipoprotein E phenotype to myocardial infarctior and mortality from coronary artery disease.

Eichner JE, Kuller LH, Orchard TJ, Grandits GA, McCallum LM, Ferr RE, Neaton JD.

Graduate School of Public Health (Departments of Epidemiology and Humai Genetics), University of Pittsburgh, Pennsylvania.

The apolipoprotein E polymorphism is a genetic determinant of low-density lipoprotein (LDL) cholesterol. Its status as a risk factor for coronary artery disease (CAD), either through a causal relation with LDL cholesterol level oı independently, is less clearly established. Data from the Multiple Risk Factoı Intervention Trial were used to examine the influence of apolipoprotein E phenotype on risk of coronary events. Of the 12,866 randomized participants 619 were studied in a nested case-control design. CAD deaths (93) and nonfa myocardial infarctions (113) were matched to 412 controls. The allele frequencies of apolipoprotein E in the white subset (epsilon 2 = 0.06, epsilon = 0.79, and epsilon 4 = 0.15) were very similar to other nonselected white American populations, and the relation of apolipoprotein E on total and LDL cholesterol was generally similar to that seen in other studies, with the epsilo 2 allele being associated with lower and the epsilon 4 allele with higher total and LDL cholesterol. Allele frequencies were not the same for patients and control subjects. The presence of epsilon 4 was associated with an increased risk of CAD that was most evident for fatal cases. There was no relation between changes in LDL cholesterol over time during the trial and apolipoprotein E phenotypes.

Publication Types:
- Clinical Trial
- Randomized Controlled Trial

PMID: 8421977 [PubMed - indexed for MEDLINE]

Exhibit 5
U.S. Patent No. 10/017,718

1: Neurology. 1995 Mar;45(3 Pt 1):555-7.

Synergistic effects of traumatic head injury and apolipoprotein-epsilon 4 in patients with Alzheimer's disease.

Mayeux R, Ottman R, Maestre G, Ngai C, Tang MX, Ginsberg H, Chun M, Tycko B, Shelanski M.

Gertrude H. Sergievsky Center, Columbia University, New York, NY 10032

The apolipoprotein-epsilon 4 allele increases the risk of Alzheimer's disease (AD), but cerebral deposition of beta-amyloid with age, a genetic mutation, c head injury may contribute to the pathogenesis of this disease. We examined the risks of AD associated with traumatic head injury and apolipoprotein-epsilon 4 in 236 community-dwelling elderly persons. A 10-fold increase in t risk of AD was associated with both apolipoprotein-epsilon 4 and a history o traumatic head injury, compared with a two-fold increase in risk with apolipoprotein-epsilon 4 alone. Head injury in the absence of an apolipoprotein-epsilon 4 allele did not increase risk. These data imply that th biological effects of head injury may increase the risk of AD, but only throug a synergistic relationship with apolipoprotein-epsilon 4.

PMID: 7898715 [PubMed - indexed for MEDLINE]

Exhibit 6
U.S. Patent No. 10/017,718

1: Neurology. 2001 Sep 11;57(5):853-7.

Apolipoprotein E epsilon 4 is associated with rapid progression multiple sclerosis.

Fazekas F, Strasser-Fuchs S, Kollegger H, Berger T, Kristoferitsch W, Schmidt H, Enzinger C, Schiefermeier M, Schwarz C, Kornek B, Reindl M, Huber K, Grass R, Wimmer G, Vass K, Pfeiffer KH, Hartung HP, Schmidt R.

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OBJECTIVE: The apolipoprotein E (APOE) polymorphism is known to imp on various neurologic disorders and has differential effects on the immune system and on CNS repair. Previous findings concerning a possible modulati of the clinical course of MS have been inconsistent, however. METHODS: I cross-sectional study, the authors investigated 374 patients with clinically definite MS and a disease duration of at least 3 years and related their clinica and demographic findings to the allelic polymorphism of the APOE gene. Th genotype distribution of patients with MS was compared with a cohort of 38 asymptomatic, randomly selected elderly volunteers. RESULTS: The author found no significant differences in the distribution of genotypes between patients with MS and controls. However, patients with MS with the epsilon4 allele (n = 85) had a significantly higher progression index of disability (0.46 +/- 0.4 versus 0.33 +/- 0.26; $p < 0.004$) and a worse ranked MS severity scor (5.1 +/- 1.9 versus 5.7 +/- 1.7; $p = 0.05$) than their non-epsilon4 counterparts, despite significantly more frequent long-term immunotherapy in epsilon4 carriers (74% versus 58%; $p < 0.007$). The annual relapse rate in epsilon4 carriers (0.87 +/- 0.56) was significantly higher than in patients with MS without an epsilon4 allele (0.71 +/- 0.47; $p = 0.03$). CONCLUSIONS: These results suggest no effect of the APOE genotype on susceptibility to MS, but indicate an association of the APOE epsilon4 allele with a more severe cours of the disease.

Publication Types:
- Clinical Trial

Exhibit 7
U.S. Patent No. 10/017,718

1: J Neurol Sci. 2001 Sep 15;190(1-2):17-20.

Association of APOE epsilon4 allele with survival in amyotrophi lateral sclerosis.

Drory VE, Birnbaum M, Korczyn AD, Chapman J.

Department of Neurology, Tel-Aviv Sourasky Medical Center, Ramat-Aviv, Israel.

APOE epsilon4 allele is associated with poorer outcome in degenerative neurological diseases. Its role in amyotrophic lateral sclerosis (ALS) is still unclear. The aim of the present study was to further analyze the association c APOE epsilon4 allele with progression and survival of ALS.One hundred consecutive ALS patients (53 males) and 133 controls were genotyped for th APOE epsilon4 allele. The association of this allele with survival to death or tracheostomy was analyzed by Kaplan-Meier survival analysis.The frequenc of the APOE epsilon4 allele in ALS patients was slightly higher (15.1%) tha in the control group (10.9%). Patients with or without an APOE epsilon4 alle had a similar age of onset and frequency of bulbar onset. There was a significant shortening of the 50% probability of survival (by 32 months) in patients carrying the APOE epsilon4 allele (p=0.03).In conclusion, carrying a APOE epsilon4 allele is a poor prognostic factor in ALS. This is compatible with a role of apolipoprotein on neuronal survival and repair.

PMID: 11574101 [PubMed - indexed for MEDLINE]

Exhibit 8
U.S. Patent No. 10/017,718

1: Ann N Y Acad Sci. 1998 Nov 30;855:738-43.

Apolipoprotein E and Alzheimer's disease. The tip of the susceptibility iceberg.

Roses AD.

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Apolipoprotein E (APOE) is a true susceptibility polymorphism of the comm form of Alzheimer's disease (AD). There are three APOE alleles (epsilon 2, epsilon 3, epsilon 4) that are universally distributed in the population with some variation in allele frequency due to racial and ethnic differences, and ar associated with different risks and age of onset distributions. In multiple studies, the positive predictive value for symptomatic possible or probable A patients who carry at least one epsilon 4 allele was consistently > 95%. Thus, early in the clinical course of dementia, when diagnoses are only 60-70% accurate, the presence of an epsilon 4 allele raises the diagnostic accuracy of AD to 95%. With the anticipation of a second major late-onset AD susceptibility locus on chromosome 12, a matrix of relative susceptibility risl in the population raises many ethical and social questions associated with preclinical prediction. The metabolism of apoE (protein) in the brain is a new and exciting area of neurobiology research made relevant by the association with AD. We have constructed transgenic animals using large human genom fragments containing human APOE on an APOE-deficit mouse background a well as homologous recombination experiments replacing mouse APOE with human APOE promoter elements. The APOE tissue elements, NOT the huma APOE gene coding sequence, is associated with the human pattern of intraneuronal apoE immunoreactivity.

Publication Types:
- Review
- Review, Tutorial

PMID: 9929679 [PubMed - indexed for MEDLINE]